

Selected New Developments in Asbestos Immunotoxicity

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Research over the past three decades has shown that the mammalian immune system can be altered by the occupational exposure of asbestos. Early clinical studies generally focused on systemic observations of immune alteration such as the number and function of peripheral lymphocytes and monocytes. More recently as the regulatory influence of local immunity in health and disease becomes more defined, immunologic changes occurring in the lung, the primary target organ of asbestos, have been significant areas of investigation. This review will focus on recent studies that examine the influence of asbestos on pulmonary immunity as well as the role of host immune competence in asbestos-related disease. — *Environ Health Perspect* 106(Suppl 1): 159–169 (1998). <http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-1/159-169rosenthal/abstract.html>

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Introduction

Occupational exposure to asbestos is widely believed to pose an increased risk for a range of pulmonary diseases and is considered an important cause of pulmonary cancer (i.e., diffuse malignant mesothelioma, bronchiogenic carcinoma). The predominant disease associated with asbestos exposure, asbestosis, is characterized by an interstitial pulmonary fibrosis commonly thought to represent the terminal phase of chronic inflammation (1,2). This inflammatory response, along with evidence of peripheral immune changes following asbestos exposure, has long implicated the pathogenesis of asbestos-related disease (3).

Unlike many industrial immunotoxins, for which relatively little clinical data are available, there exists a comparatively large literature database documenting the direct and indirect effects of asbestos on the human immune system, although the specific role of these changes in eliciting asbestos-related disease is unclear. In numerous clinical studies both humoral and cellular immunity have been targets of asbestos toxicity (4–12). For example, studies performed in the late 1980s by researchers at the Mount Sinai School of Medicine, New York (4) used peripheral blood lymphocytes in their examination of T cells, T-cell subsets, and natural killer (NK) cells in 118 healthy control subjects and compared these data to those obtained from 20 patients with clinically diagnosed malignant mesothelioma and 375 long-term asbestos workers without neoplasia. These studies showed that whereas the absolute numbers of total T cells and T-helper (Th) cells were normal in asbestos workers without neoplasia, these cells were significantly reduced in patients with neoplasia. T-suppressor (Ts) cells, on the other hand, remained unchanged in the mesothelioma population but were significantly elevated among the asbestos workers without neoplasia. This imbalance in T-cell subsets resulted in a marked reduction in Th to Ts ratios in mesothelioma patients and in asbestos workers.

Similar to its effects on cellular immunity, a number of reports demonstrate asbestos-associated changes in humoral immunity, as manifested by increased circulating levels of immunoglobulins (6), including auto-antibodies and rheumatoid factors (8,10–12). More recently, studies have experimentally demonstrated the importance of certain components of the complement system (13,14) and the importance of fiber deposition and interstitial translocation to the ultimate pathology associated with asbestos-related inflammation. Specifically, following transepithelial passage, fiber-induced activation of local complement can generate potent chemoattractants that likely serve as important initiators of inflammatory events (13,14). These studies were recently reviewed by Warheit and Hesterberg (15). The purpose of this paper is to summarize recent key developments in our understanding of the complex interactions of asbestos with cells of the immune system.

Influence of Asbestos Exposure on Nonspecific Immunity: Natural Killer Cells

Natural killer cells are a unique lymphocyte population with the ability to rapidly lyse tumor cells independent of major histocompatibility complex gene products (16) and are thought to be the first line of defense against cancer cells or virally infected cells (17,18).

In addition to the ability of asbestos fibers to substantially increase the risk of pulmonary malignancy, clinical studies have shown that NK cells isolated from peripheral blood of patients with asbestosis have impaired NK cell activity, thus leading to speculation that such suppression may have a causative or multiplicative effect on the risk for lung cancer in these individuals (4,5). To date, it is not known if this immune deficit precedes or is the consequence of neoplastic and/or fibrotic development or if the immunologic perturbations are an epiphenomena unrelated to the pathogenesis of asbestos-induced cancer.

Considering the established role of NK cells in tumor immunosurveillance, an adequate understanding of asbestos-induced suppression of NK cell activity may provide insight into the mechanism(s) by which asbestos exerts its carcinogenicity. Unfortunately the early clinical assessments of peripheral lymphocytes provide only an

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Abbreviations used: AM, alveolar macrophage(s); DMTU, dimethylthiourea; FIGF, fibroblast inhibitory growth factor; IGF, insulinlike growth factor; IL, interleukin; MCP-1, macrophage chemotactic protein 1; MIP, macrophage inflammatory protein; NF, nuclear factor; NK, natural killer; PDGF, platelet-derived growth factor; PMN, polymorphonuclear neutrophil; ROS, reactive oxygen species; SCID, severe combined immunodeficiency disease; TGF- α , transforming growth factor alpha; Th, T-helper; TMTU, tetramethylthiourea; TNF, tumor necrosis factor; Ts, T-suppressor.

indirect indication of the immunologic status of the host lung, as pulmonary tissue itself possesses an effective localized system of immunologic components, including the alveolar macrophage (AM) and interstitial lymphocytes. Thus, while circulating NK cells may play a role in controlling the development of pulmonary cancer, the interstitial pulmonary NK cell is a more likely candidate for the local control of neoplastic development in the lung. Within the interstitial pulmonary lymphocyte population, lung NK cells exist in great quantity, with an effective percentage exceeding that of any other organ of the body on a per-lymphocyte basis (19), a characteristic that may have evolved in response to the numerous carcinogenic challenges of inspired air. Although the specific role of these cells in asbestos-induced lung disease remains unknown, a reasonable hypothesis maintains that these lung-localized cells protect the host from altered phenotype expression(s) that may arise during the pathogenesis of asbestosis. In support of this role, recent studies have shown the ability of NK cells to lyse tumors of mesothelial origin (20).

In light of the previously reported observations of decreased number and function of circulating NK cells in asbestos-exposed workers, studies were conducted to examine the influence of inhaled asbestos on interstitial NK number and function in C57BL/6 mice (21). In these studies C57BL/6 mice were exposed to chrysotile asbestos (~13.3 mg/m³) for 3 hr/day for 3 days and animals sacrificed at 7, 28, and 56 days postexposure. Functional assessment of the interstitial NK population was determined by evaluation of their ability to lyse target YAC-1 cells. As in Table 1, the ability of pulmonary NK cells to lyse target cells was significantly suppressed in asbestos-exposed mice on days 7 and 56 postexposure compared to air-exposed animals. Although cytotoxicity appears decreased in asbestos-exposed animals on day 28, the degree of suppression did not reach statistical significance. This suppression in target cell-mediated cytotoxicity by NK cells isolated from asbestos-exposed mice correlated to some extent with the percentages of NK cells recovered from these mice, which were below those recovered from air controls at all time points examined (Table 2). The effect of asbestos on pulmonary NK cells appears relatively specific, as the relative and absolute numbers of pulmonary T cells (Table 2) and splenic NK cells (data not shown) were generally unaffected at these times.

It is often difficult to sort out the causative forces occurring soon after asbestos exposure because of inflammation-related cell trafficking. Thus, time points taken long after exposure are often useful in sorting out effects that may or may not be related to the presence and activity of inflammatory cells. Asbestos inhalation in this study, as in others, was immediately followed by a marked neutrophil accumulation that had not resolved at the first measured time point, day 7 postexposure (Table 3). However, this dynamically changing cell population appeared to normalize 28 days postexposure and is therefore unlikely to account for the observation of suppressed NK cell numbers (via dilution) on days 28 and 56 or the impaired functional activity on day 56. Thus, it appears that in addition to the decreased levels of circulating NK cells previously reported in humans (4,5), the number of local or interstitial NK cells in pulmonary tissue may also be altered following exposure to asbestos. Further studies will be necessary to understand the importance of this altered cellular distribution in local immune regulation as well as in the pathogenesis of asbestos-related disease.

Table 1. Effect of asbestos inhalation on interstitial natural killer cell-mediated cytotoxicity.

Group	Days postexposure	Cytotoxicity, %
Air	7	22 ± 5
Asbestos ^a	7	9 ± 3*
Air	28	18 ± 6
Asbestos ^a	28	13 ± 4
Air	56	19 ± 2
Asbestos ^a	56	10 ± 3*

^aWhole-body asbestos exposure to C57BL/6 mice for 3 hr/day for 3 consecutive days at a concentration of ~13.3 mg/m³. Each value represents the mean ± SD of 6 to 12 mice per group. *Significantly different ($p < 0.05$) from corresponding air control groups using Student's *t*-test.

Influence of Asbestos on Nonspecific Immunity: Lung Macrophages

Pulmonary macrophages seem to play a central role in asbestos-induced chronic inflammation and fibrosis in the lung (22). Anatomically, this heterogeneous group of cells can be distinguished by their pulmonary distribution, i.e., airway, alveolar, interstitial, intravascular, and pleural. These specific cells have different morphologic features and most likely exhibit different responses on interactions with particulates (23). AM represent the first line of defense in the alveolar region of the lung and their expansion in the lung is a typical characteristic of asbestos exposure in both humans and experimental animals. For example, increased numbers of activated AM have been found in the lower respiratory tract of humans chronically exposed to high concentrations of asbestos fibers (24). Inhalation of asbestos by rats demonstrated a 10-fold increase in the number of AM and a 3-fold increase in interstitial macrophages in the lung 2 days after asbestos exposure (22). Total macrophage numbers in the lung may increase by a

Table 2. Effect of asbestos inhalation on interstitial natural killer cells and interstitial T cells.

Group	Days postexposure	NK 1.1	Thy 1.2
Air	7	19.4 ± 4.1	52.1 ± 10.6
Asbestos ^a	7	7.0 ± 0.6*	46.4 ± 8.4
Air	28	22.7 ± 2.0	50.3 ± 3.6
Asbestos ^a	28	13.9 ± 2.9*	57.6 ± 5.4
Air	56	22.7 ± 1.8	56.4 ± 5.3
Asbestos ^a	56	17.3 ± 1.3*	52.3 ± 5.0

^aWhole-body asbestos exposure to C57BL/6 mice for 3 hr/day for 3 consecutive days at a concentration of ~13.3 mg/m³. Each value represents the mean ± SD of 6 to 12 mice per group. *Significantly different ($p < 0.05$) from corresponding air control groups using Student's *t*-test.

Table 3. Differential analysis of nylon wool-purified interstitial pulmonary cells in asbestos- and air-exposed mice.

Group	Days postexposure	Recovered cells, %		
		Lymphocytes	Neutrophils	Macrophages
Air	7	86 ± 3	12 ± 5	3 ± 2
Asbestos ^a	7	68 ± 4*	29 ± 5*	4 ± 1
Air	28	89 ± 6	10 ± 5	1 ± 1
Asbestos ^a	28	90 ± 4	8 ± 4	2 ± 1
Air	56	87 ± 2	7 ± 3	5 ± 4
Asbestos ^a	56	87 ± 2	10 ± 3	4 ± 1

^aWhole-body asbestos exposure to C57BL/6 mice for 3 hr/day for 3 consecutive days at a concentration of ~13.3 mg/m³. Each value represents the mean ± SD of 6 to 12 mice per group. *Significantly different ($p < 0.05$) from corresponding air control groups using Student's *t*-test. Note: nylon wool would be expected to remove the majority of macrophages.

variety of mechanisms including migration of blood monocytes, local proliferation of the AM (25,26), or asbestos-induced generation of chemotaxins such as certain complement components (13,14). The role of the other locally produced factors such as monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein (MIP)-1 α and -1 β , known chemoattractants for monocytes (27), is not yet well characterized for the asbestos-induced inflammatory response. In addition, the proliferative response of the AM population seen after silica and asbestos exposure may be induced by colony-stimulating factors (28) and is believed to be associated with the formation of multinucleated giant cells (29), a prominent feature in the chronic stage of asbestosis and silicosis (30,31). In addition, AM produce insulinlike growth factor (IGF)-1 and express its receptor, thus creating an environment for the autocrine stimulation of AM proliferation (32,33).

AM provide dual defense of the lower respiratory tract, including phagocytosis and removal of inhaled fibers and triggering local immunologic events that may be protective. As a phagocytic cell the AM helps clear the lung of inhaled particles. Evaluation of AM from humans and animals exposed to high concentrations of asbestos show phagocytized fibers in numerous AM (24). Several lines of *in vitro* evidence suggest that AM, unlike the polymorphonuclear neutrophil(s) (PMN), which also increase in asbestosis, can reduce asbestos-induced epithelial cell injury through initiation of key repair processes (34,35).

Activated AM are abundant sources of proinflammatory mediators and growth factors with important roles in the pathogenesis of inflammatory and fibrotic processes in the lung. The oxygen metabolites and eicosanoids released from AM during the phagocytosis of asbestos fibers are well documented (24,36,37). Overwhelming evidence shows that the pulmonary macrophage can be stimulated by asbestos to express and secrete multiple inflammatory cytokines, chemokines, and growth factors participating in both auto-crine and paracrine stimulation of resident lung cells and blood cells (Table 4). Tumor necrosis factor (TNF) α has received considerable attention in this regard, as several lines of evidence suggest that it plays a significant role in fiber-induced lung disease. AM secrete TNF- α after *in vivo* inhalation, *in vitro* exposure to asbestos fibers (38–40), and AM from asbestosis patients (41) or patients with history of chronic asbestos exposure (38)

demonstrate an increase in TNF- α message expression and secretion. Additionally, excess TNF- α correlates with development of fibrosis in animal models of asbestosis (42,43). Interestingly, the long fibers, which are generally considered more carcinogenic and fibrogenic than short fibers of similar diameter (44), stimulated a greater release of TNF- α than the short samples (45). TNF- α administered *in vivo* can elicit many of the responses associated with asbestosis, including proliferation of fibroblasts (46,47), stimulation of extracellular matrix proteins (41), and elicitation of inflammatory cells via enhanced adhesion molecule expression and the production of chemokines (48). Consistent with a role for TNF in particulate-related lung disease, studies have shown that the administration of antibodies to TNF- α prevents collagen deposition induced by silica, another fibrogenic material (49).

In light of their propensity to elicit potent inflammatory cytokines, it is not surprising that AM are thought to be involved in the recruitment of neutrophils to the lung. AM from subjects with asbestosis release neutrophil chemotactic factors (50), which may be a secondary response to TNF- α and directly stimulated by asbestos itself. The presence of neutrophils is a frequent finding in animal models of asbestosis (22) and humans occupationally exposed to asbestos (51,52). Elevated neutrophils are often associated with lung damage, as they are potent producers of reactive oxygen and proteolytic enzymes, both with the potential to destroy respiratory tissue. Human interleukin (IL)-8 and its mouse analog MIP-2, chemokines from the α -intercrine family, are the most stable and potent chemotactic factors for PMN in the lung (53). Further evidence for fiber-induced induction of inflammatory cytokines can be seen in recent studies showing that silica induces MIP-2 from AM (54) and asbestos stimulates IL-8 production from lung epithelial cells (55) and human AM (56).

Table 4. Asbestos-induced cytokines and growth factors produced by pulmonary macrophages.

Cytokines/growth factors	Reference
TNF- α	(35,38,40–42,120)
IL-1 α /IL-1 β	(39,120,137)
IL-6	(139)
TGF- α	(63)
PDGF	(58)
IGF	(32)
Fibroblast growth factor	(138)

There is now substantial evidence that the pulmonary macrophage may be involved in tissue remodeling in the lung. AM from individuals with asbestos exposure spontaneously release large amounts of fibronectin (24), a potent chemoattractant for lung fibroblasts (57). Additionally, asbestos-induced secretion of platelet-derived growth factor (PDGF) (58), a potent mitogen and chemoattractant for mesenchymal cells (59), has been described for AM as well as many other cells in the lung; the mechanisms of the expression of PDGF isoforms have been studied extensively (60,61). Studies on the activation of interstitial macrophages have revealed that they release fibroblast growth factors, including PDGF, which may be particularly important in light of their proximity to the target interstitial fibroblast (62). Asbestos-stimulated pulmonary macrophages are also a source of additional fibroblast growth factors such as IGF-1 (32) and transforming growth factor α (TGF- α) (63), a growth factor with potent mesenchymal and epithelial mitogenic activity expressed in the lung at sites of asbestos deposition (64,65). It is likely that this growing list of growth-promoting factors is influential in both asbestos-induced fibrosis as well as in the epithelial repair processes in the lung.

Influence of Asbestos Exposure on Nonspecific Immunity: Epithelial Cells

Recently, pulmonary parenchymal cells have been recognized as participants in normal immune regulation as well as in various pulmonary disease states. For example, in addition to PDGF, pulmonary fibroblasts secrete MIP-1 α (66), a peptide with leukocyte-activating and chemotactic properties, as well as IL-8, a potent chemoattractant (67). Similarly, pulmonary epithelial cells can produce IL-8 and MCP-1 (68). Considering that intact alveolar space is lined by epithelial cells, direct asbestos–epithelial cell contact without intervening AM is likely to occur. Type II epithelial cells alone account for over 15% of the cells in the distal lung (69) and their direct proximity to inspired air make their functional properties an integral consideration in understanding the pathophysiology of asbestos-induced lung disease. Furthermore, pulmonary neutrophils, a consistent finding in asbestos-exposed workers (52,53), correlate with the duration of asbestos exposure (52). They have been strongly implicated in the pathogenesis of asbestos-induced fibrosis through release of reactive oxygen intermediates and proteases (70,71), with

resulting tissue damage. Prompted by these observations, our laboratory at the National Institute of Environmental Health Sciences (Research Triangle Park, NC) initiated studies to assess the direct effects of asbestos fibers on chemokine secretion in pulmonary epithelial cells.

In these studies the human pulmonary type II epithelial cell line A549 cultured in the presence of either chrysotile or crocidolite asbestos induced a dose-dependent increase in IL-8 release (Figure 1, Table 5). Of the two fibers crocidolite was slightly more potent than chrysotile, resulting in a 20-fold increase in IL-8 secretion when compared with untreated cells. The chemotactic activity for human neutrophils was also examined to establish whether immunoreactive IL-8 detected in culture supernatants was biologically active. As in Figure 2, culture supernatants from asbestos-exposed A549 cells induced a marked chemotactic response that was inhibitable by > 60% after incubation with anti-IL-8 antibody, demonstrating that most of this chemotactic activity is likely to be bioactive IL-8. Wollastonite and titanium oxide, poorly fibrogenic agents, did not elicit IL-8 when tested at significantly higher concentrations than used for asbestos (Figure 3), suggesting that specific physicochemical properties of the fibers play a key role in this response. Factors such as fiber size, surface charge, charge density, surface adsorption, reactive surface sites, and chemical composition have all been suggested as contributors to asbestos-associated biologic activity (72).

The membrane signaling events responsible for IL-8 production in pulmonary epithelial cells possess some specificity, with IL-1 and TNF inducing large amounts and lipopolysaccharide showing little or no stimulatory capacity (67). The role of membrane signaling events requiring actin-myosin filament assembly such as phagocytosis (73) was also examined, specifically comparing IL-8 release stimulated by TNF and asbestos. In these studies cytochalasin D, an inhibitor of microfilament assembly, did not influence TNF-mediated IL-8 release but substantially inhibited asbestos-mediated IL-8 release. This finding suggests that in contrast to proinflammatory cytokine-mediated signaling, asbestos-mediated signaling requires the assembly of actin-myosin filaments.

These data suggest that epithelial cells in addition to macrophages and fibroblasts may be important effector cells in the immunopathogenesis of asbestos-associated diseases and in particular in the neutrophilic

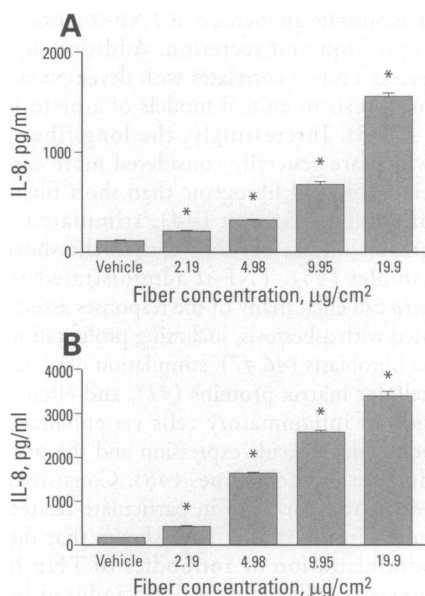


Figure 1. IL-8 release from A549 cells after addition of (A) chrysotile or (B) crocidolite asbestos. A549 cells were plated at 3×10^5 /well in 24-well microtiter plates and incubated overnight to achieve confluence. Eighteen hours after plating, culture media was decanted and replaced with 1 ml fresh media. Asbestos fibers were suspended in F12 media with serum and added to cultures in 20-µl aliquots. Cells were incubated for an additional 18 hr and IL-8 present in cultures was quantitated using a commercially available enzyme-linked immunosorbent assay (ELISA) system. * $p \leq 0.05$. Data adapted from Rosenthal et al. (55).

infiltration that is frequently observed following asbestos exposure. Nevertheless, it is important to remember that pulmonary epithelial cells do not exist singularly and the production of TNF and IL-1 by other lung cells such as the AM can influence cytokine production from epithelial cells. The relative importance of these varying sources has yet to be defined.

The Influence of Asbestos on Specific Immunity

One of the most consistent findings in individuals chronically exposed to asbestos is an elevation in serum immunoglobulins (IgA, IgG, IgM, IgE) and mucosal (salivary) IgA and the presence of autoantibodies (antinuclear antibody, rheumatoid factor) (7,8,9). The incidence of autoantibodies in asbestos-exposed workers with radiographic abnormalities varies widely and ranges from 3 to 28% for antinuclear antibody and from 10 to 38% for rheumatoid factor (7,8,10,12,74). Although elevated levels of IgG and IgM appear to correlate with chest radiographic classification of pneumoconiosis, no correlation exists between

Table 5. Lactate dehydrogenase release.

Asbestos, µg/cm²	U/liter
Chrysotile	
0	16.1 ± 0.7
2.49	17.9 ± 0.3
4.98	19.0 ± 2.2
9.95	22.1 ± 1.4
19.9	26.6 ± 1.1
Total release	132.1 ± 4.5
Crocidolite	
0	17.2 ± 0.5
2.49	19.9 ± 1.4
4.98	22.1 ± 1.9
9.95	28.5 ± 1.6
19.9	39.7 ± 0.4
Total release	132.1 ± 4.5

LDH, lactate dehydrogenase release. LDH was used as a measure of cellular damage. Values are expressed as mean ± SD of quadruplicate cultures. Data adapted from Rosenthal et al. (55).

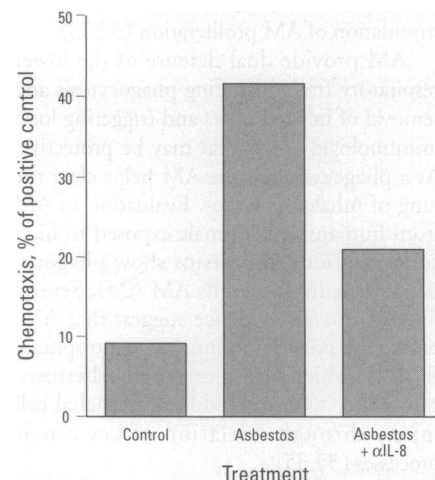


Figure 2. Supernatants from A549 cells previously stimulated for 18 hr with 6.0 µg/ml crocidolite asbestos ± 30 min incubation with 1:100 dilution of polyclonal anti-IL-8 antiserum. A mean of 302 neutrophils/high-powered field was observed with the positive control (10^{-7} M formyl-met-len-phe). Values are representative of duplicate experiments. Data adapted from Rosenthal et al. (55).

autoantibody production or serum immunocomplexes and the severity of asbestosis (9). Regardless of its correlation with disease state, the presence of autoantibodies, elevated serum immunoglobulins, and the detection of serum immunocomplexes are indicative of B-lymphocyte hyperactivity in asbestos-exposed workers. *In vitro* studies using human cell lines have shown that both chrysotile and crocidolite asbestos can complex with immature B lymphocytes and stimulate cellular proliferation (75), and *in vivo* studies in experimental animals

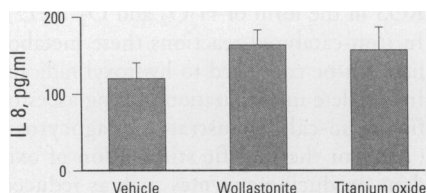


Figure 3. Effects of nonfibrogenic dusts on the release of IL-8 from A549 cells. Wollastonite and titanium oxide were added at a concentration of 80 $\mu\text{g/ml}$ and IL-8 quantitated using a commercially available ELISA system. Values are expressed as mean \pm SD of quadruplicate cultures. Data adapted from Rosenthal et al. (55).

injected with crystalline asbestos have shown a boost in serum γ -globulin levels (8). Furthermore, an adjuvantlike effect of asbestos in rodents has been demonstrated and appears related to an AM membrane surface-related structure (76). Such an asbestos-related neoantigen may play a part in nonorgan-specific autoantibodies in some patients with asbestosis. Asbestos exposure is associated with an increase in Ia expression, a mediator of macrophage-lymphocyte communication on the surface of AMs, which may induce these cells to stimulate self-targeting lymphocytes (77). Alternatively, it has been suggested that asbestos-induced B-cell overactivity may be related to decreased suppressor cell activity and/or numbers previously reported in asbestos-exposed workers.

Although the effects of asbestos on humoral immunity can be considered hyperactive, the clinical studies to date portray a significantly depressed cell-mediated immune response in asbestos-exposed individuals. A clear relationship between defective T-cell functions and the fibrotic response in asbestosis has been demonstrated (7,78,79), with the intensity of the decrease paralleling the severity of the disease (79). Asbestos-exposed patients with impaired T-cell responses have more severe fibrotic abnormalities (as determined by chest radiography) than those with normal responses, and patients with increases in bronchoalveolar lymphocytes present with less physiologic impairment than those with increased neutrophils and eosinophils (78,80–82). Furthermore, the unexpected longevity of a patient with malignant pleural mesothelioma was associated with normal lymphocyte surface markers and functions in contrast to mesothelioma patients who terminally progressed with suppressed numbers and function of T and B lymphocytes (83). However, in one study no association was found between impaired

cellular immunity and asbestos-associated malignancy in eight of ten patients with asbestos-associated pleural mesothelioma and without lung fibrosis (78), indicating that impaired T-cell function is an unlikely finding in all asbestos-associated malignancy. In patients with radiographic evidence of parenchymal asbestosis, both the relative and absolute number of circulating T lymphocytes were significantly depressed compared to control (7). This T-cell deficit was associated with suppressed mitogen-induced lymphocyte proliferation, cutaneous anergy to dinitrochlorobenzene, and depressed delayed-type hypersensitivity to several antigens (7,84–86). *In vitro* studies with human peripheral blood lymphocytes have shown that asbestos-depressed phytohemagglutinin A induced lymphoproliferation in a manner that was only partially related to cytotoxicity (87,88). Reduced CD4:CD8 ratios in bronchoalveolar lavage fluid have been described in asbestos-exposed workers with and without radiologic evidence of asbestosis; this finding has correlated with pleural thickening (89–91). A local excess of T_s cells or depletion of T_h cells might result from nonspecific activation of the immune system secondary to AM activation by asbestos. Although unlikely, reduced CD4:CD8 ratios may result from nonspecific direct damage to lymphocytes by asbestos fibers. It is conceivable that the loss of T_s cell regulation could explain the B-cell reactivity and that the neoplastic cell-transforming properties of asbestos might act in concert with this immune-deficient host. In addition one may speculate that the previously described effects on NK cells may be related to the observation that among asbestos-exposed workers with depressed T-cell functions there is an increase in the number of the effector T_s (Leu²⁺ Leu⁸⁻) subsets that regulate both the T_h:T_s ratio as well as B- and NK cell activity (92).

Although T cells are clearly a target of asbestos exposure, it is important to note that asbestos-exposed individuals present myriad patterns of T-cell subset alterations, including an increased CD4:CD8 ratio (93). Interestingly, chrysotile asbestos interferes with phytohemagglutinin or concanavalin A-induced release of fibroblast inhibitory growth factor (FIGF) from peripheral blood lymphocytes *in vitro* (94). Thus, while FIGF may regulate the extent of connective tissue proliferation during normal repair process, suppression of it by asbestos may contribute to excessive fibroblast accumulation and subsequent fibrosis.

Although most immunologic alterations are generally found in patients with radiologic evidence of asbestosis, two studies have reported leukopenia in presumably healthy individuals with occupational asbestos exposure (95,96). Thus, it is difficult to establish whether the cellular defects precede or are the consequences of the development of fibrosis. To answer this question, our laboratory evaluated asbestos-induced inflammatory and fibrotic responses in lungs of immunodeficient mice (Balb/c nu/nu) and severe combined immunodeficient (SCID) (C₃H) mice, immunologically normal mice of the same genetic background, and immunodeficient mice reconstituted with syngeneic T lymphocytes (97). In these studies, increases in bronchoalveolar lavage cell numbers occurred in asbestos-treated immunodeficient mice compared to asbestos-treated immunocompetent mice or immunodeficient mice that were reconstituted with crude and purified T lymphocytes (Table 6). Differential analysis of the collected cells portrayed a predominantly neutrophilic infiltrate, which correlated with increased levels of LTB₄ and PGE₂. Both asbestos-treated athymic and SCID mice showed a significant increase in total lung

Table 6. Effect of chrysotile asbestos on total cells and cell differential in pulmonary lavage fluid 6 weeks after exposure in immunocompetent, SCID, or reconstituted SCID mice.

Strain/treatment	Total cells, $\times 10^5$	Macrophages, %	Neutrophils, %
C ₃ H/air	0.93 \pm 0.10	89.5 \pm 1.6	9.4 \pm 1.2
C ₃ H/asbestos	1.93 \pm 0.08 [#]	83.9 \pm 5.2	9.6 \pm 3.6
SCID/air	1.28 \pm 0.17	94.6 \pm 0.9	4.6 \pm 0.6
SCID/asbestos	5.61 \pm 1.43 [#]	60.6 \pm 2.0 [#]	37.8 \pm 1.8 [#]
SCID-L/asbestos	1.72 \pm 0.12 ^{#*}	88.0 \pm 3.4 [*]	10.4 \pm 3.0 [*]
SCID-T/asbestos	1.76 \pm 0.20 [*]	91.6 \pm 2.6 [*]	7.2 \pm 2.2 [*]

Each value represents the mean \pm SE of three to seven determinations. Statistical analysis was performed using the Student's *t*-test. [#] $p < 0.05$ compared to asbestos-exposed SCID mice. ^{*} $p < 0.5$ versus asbestos-exposed SCID mice. SCID-L are SCID mice reconstituted with 2×10^7 unfractionated lymphocytes; SCID-T are SCID mice reconstituted with 1×10^7 purified T lymphocytes. SCID mice were of C₃H genetic background. Table adapted from Corsini et al. (97).

hydroxyproline when compared to asbestos-treated immunocompetent mice (Table 7).

Consistent with this, lung hydroxyproline was reduced in asbestos-exposed SCID mice reconstituted with T lymphocytes and conversely increased in T-cell depleted Balb/c mice (data not shown). Histopathologic assessment demonstrated that asbestos exposure was associated with both cellular and fibrogenic inflammatory responses, with asbestos-exposed SCID mice presenting with a more severe cellular response than similarly exposed normal mice (Table 8). Taken together, these data indicate that T cells influence asbestos-induced lung damage by minimizing both the inflammatory and fibrotic responses and taken together are consistent with a protective role for T lymphocytes in asbestosis. It is likely that the more severe neutrophil infiltration and the greater retention time of these cells in the lung of immunodeficient mice causes more extensive lung injury.

Table 7. Effect of chrysotile asbestos on total lung hydroxyproline 6 weeks postexposure.

Strain/treatment	Hydroxyproline, mg/lung
C ₃ H/air	224.2 ± 6.2
C ₃ H/asbestos	280.8 ± 15.7 [#]
SCID/air	235 ± 6.6
SCID/asbestos	482.9 ± 22.5 [#]
SCID-L/asbestos	387.8 ± 19.9 ^{**}
SCID-T/asbestos	413.5 ± 38.6 [#]

Each value represents the mean ± SE of three to seven determinations. Statistical analysis was performed using the Student's *t*-test. [#]*p* < 0.05 compared to air-exposed C₃H or air-exposed SCID mice. ^{**}*p* < 0.05 significantly different versus asbestos-exposed SCID mice. Table adapted from Corsini et al. (97).

Table 8. Histologic evaluation of normal C₃H, SCID, and reconstituted SCID mice 6 weeks after asbestos exposure: incidence and severity of pulmonary lesions.

Strain/treatment	Incidence ^a	Severity ^b
C ₃ H/air	0/8	—
C ₃ H/asbestos	4/9	1.0
SCID/air	0/10	—
SCID/asbestos	8/8	2.1
SCID-L/asbestos	10/10	1.2
SCID-T/asbestos	8/8	1.2

^aNumber of mice affected/number evaluated. ^bSeverity of collagen deposition was based on evaluation of a single Trichrome-stained section from each animal. All slides were evaluated initially to determine the maximal extent of the lesion then reevaluated and semi-quantitatively graded on a scale of +1 (minimal) to +3 (maximal). This grading was based on the number of inflammatory foci in the section that contained increased collagen, as well as the amount of collagen present in individual foci. Combined experiments are shown. Table adapted from Corsini et al. (97).

Based on these experimental studies and the above described human observations, solid evidence supports the notion that impaired cell-mediated immunity may represent a predisposing factor in asbestos-induced fibrosis. Further studies are necessary to determine the specific T-cell mediators that are critical to the regulation of asbestos-induced diseases.

The Role of Iron and Reactive Oxygen Species in Asbestos Immunotoxicology

A number of studies have shown that asbestos-induced pathophysiologic responses are associated with generation of various reactive oxygen species (ROS) such as superoxide anions (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (*OH). Asbestos may initiate the formation of such species through two principal mechanisms: via iron-catalyzed reactions such as the Haber–Weiss reaction or by activation of the respiratory burst activity inherent to phagocytic cells (98). The fiber itself is rich with physical and chemical properties that alone can initiate substantial reactivity with its surrounding environment. Asbestos fibers are composed of numerous hydrated silicates rich in negative charges, with a large capacity to complex transition metals on their surface (99,100). Iron is the basic transition metal on the fiber surface and is present at concentrations proportional to the density of acidic functional groups (101,102). Iron catalyzes the generation of high ROS from H₂O₂ and O₂⁻ through Fenton and Haber–Weiss reactions (103,104). Production of highly reactive hydroxyl radicals by asbestos fibers has been shown in cell-free chemical systems (105,106) as well as in lungs of animals exposed to asbestos via intratracheal installation (107). As asbestos fibers can easily penetrate cells (1) they can introduce iron into the cytosol and trigger the generation of hydroxyl radicals in the vicinity of key membrane or intracellular molecules. Studies demonstrated recently that asbestos may complex intracellular iron sources and this additional iron may increase the reactive lifetime of the fibers (108,109). In this respect asbestos bodies removed from human lung at autopsy have contained on their surface redox-active iron capable of catalyzing DNA single-strand breaks (110).

An additional mechanism for the induction of oxidative stress by asbestos is through the activation of macrophages and polymorphonuclear leukocytes to release

ROS in the form of H₂O₂ and O₂⁻ (111). In iron-catalyzed reactions these metabolites can be converted to hydroxyl radicals. Incomplete internalization of long asbestos fibers, so-called frustrated phagocytosis (112), or the specific stimulation of oxidant-producing enzymes such as reduced nicotinamide adenine dinucleotide phosphate oxidase (113) may together account for the increase in oxygen metabolites released from phagocytic cells following asbestos exposure. Oxygen free radical production may not be restricted only to phagocytic cells because epithelial cells, widely available to inhaled fibers at the alveolar surface, are also capable of releasing oxygen species (114).

Asbestos-initiated oxidative reactions can effect vital cellular macromolecules such as DNA, lipids, and proteins, leading to cell and nuclear damage, lipid peroxidation, and other events associated with cell death (108,115–117). Oxidative stress may not be terminally destructive but in moderate levels can initiate the synthesis of proteins associated with cellular physiologic or pathophysiologic responses. For example, ROS modulate events in the signal transduction cascade through an effect on protein phosphorylation (118,119). Recent studies have shown a link between asbestos-induced oxidative stress and activation of regulatory proteins involved in asbestos-mediated diseases in the lung, suggesting the existence of a cytokine network as an obligatory part of asbestos fiber–cell interactions. TNF-α released from AM is likely a key mediator in this process. Iron-catalyzed oxidative radicals have been involved in asbestos-induced signaling of TNF-α expression and secretion (120). A number of studies evaluating the role of iron and the production of ROS in asbestos toxicity utilize the potent iron chelator desferrioxamine. For example, treatment of asbestos fibers with desferrioxamine effectively chelates ferric ions from the fiber surface, decreases the ability of asbestos to generate OH radicals (100), and decreases the ability to induce cell toxicity (121), lipid peroxidation (122,123), and DNA damage (124). Pretreatment of asbestos fibers with desferrioxamine or treatment of AM with asbestos in the presence of desferrioxamine markedly diminishes the ability of the fibers to stimulate TNF-α production (Figure 4). Similar inhibition of an asbestos-induced TNF-α response was demonstrated by membrane-permeable hydroxyl scavengers such as dimethylthiourea (DMTU),

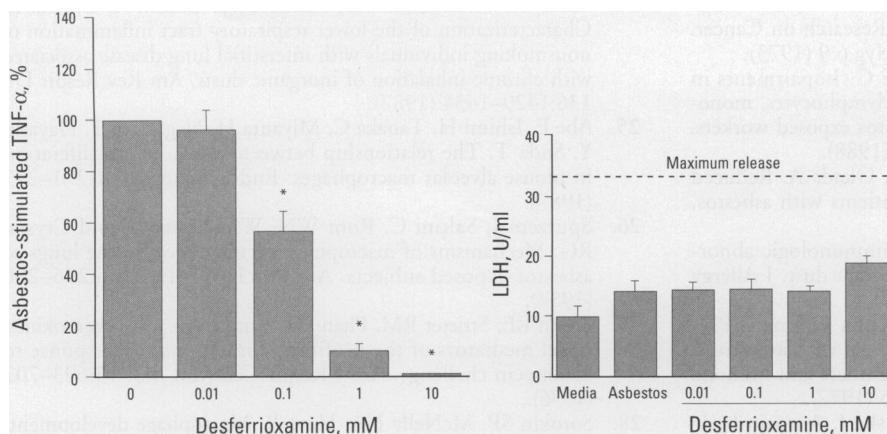


Figure 4. Effect of desferrioxamine on asbestos-induced TNF- α release by rat alveolar macrophages. AM were cultured with desferrioxamine and crocidolite asbestos (50 μ g/ml). Results are presented as the percentage of the TNF response to asbestos alone. Values represent mean \pm SE; $n = 4$ experiments. Data adapted from Simeonova and Luster (120). * $p \leq 0.05$.

tetramethylthiourea (TMTU), and dimethyl sulfoxide. Additionally, TNF- α secretion from AM is stimulated by H_2O_2 or glucose-glucose oxidase, generating H_2O_2 ; this response is increased in the presence of ferrous sulfate, which catalyzes hydroxyl radical generation through Fenton reaction.

Asbestos-induced oxidative stress is also involved in the stimulation of cytokine responses from pulmonary epithelial cells (125,126). Membrane-permeable hydroxyl scavengers significantly attenuate asbestos-activated IL-8 and IL-6 gene expression in pulmonary epithelial cells. Free radical generating systems induce IL-8 and IL-6 secretion similar to asbestos. Using antioxidants, transient transfection assays using an IL-8 promoter construct linked to a Chloramphenicol acetyltransferase reporter gene revealed that asbestos-mediated redox-oxidative intracellular changes are involved in the IL-8 promoter activation (Figure 5).

Cytokine gene transcription and expression are generally associated with the modulation of gene promoter regions by sequence-specific binding of proteins, referred to as transcription factors. Nuclear factor (NF)- κ B is a transcription factor controlling the gene expression of many inflammatory cytokines including TNF- α , IL-1 α , IL-8, and IL-6 (127–129). There is increasing evidence that the redox-oxidative stage of the cell can play a role in NF- κ B activation (130,131). ROS may serve in the posttranslational modification of NF- κ B complex including the phosphorylation and proteolysis of the inhibitory protein I κ B, resulting in release of active DNA binding complex (132). Asbestos causes hamster tracheal epithelial cells to generate nuclear

proteins that bind to the NF- κ B consensus DNA sequences. Preexposure of cells with *N*-acetyl-L-cysteine, a precursor of the antioxidant glutathione, ameliorates DNA-binding activity (133). Furthermore, asbestos fibers activate nuclear protein binding to the binding sites of specific inflammatory cytokine genes, specifically stimulating nuclear protein binding to the NF- κ B and NF-IL-6 cognate regulatory elements located in the promoter regions of IL-6 and IL-8 (125,126). For maximal expression of many inflammatory mediators, NF- κ B binding occurs simultaneously with NF-IL-6 transcription factor (134–136). Nuclear protein binding to both NF- κ B and NF-IL-6 binding sites is diminished by TMTU, an intracellular hydroxyl scavenger. Thus, asbestos-induced changes in intracellular oxidative-redox state appear to activate both NF- κ B and NF-IL-6 binding, which coordinately may regulate IL-8 or IL-6 expression in lung epithelial cells. Thus, asbestos fibers, by the presence of iron on their surface, may induce generation of oxidizing species that modulate the intracellular redox-oxidative state, which contributes to activation of transcription factors such as NF- κ B and NF-IL-6 and the subsequent stimulation of inflammatory cytokines.

Conclusion

Asbestos has proven to be an important immunotoxicant with effects on both systemic and local immunity. In fact few, if any, immunologic cells appear to be spared by asbestos exposure, either through direct effects or as a result of the hosts' protective response to exposure. Although recent studies have begun to shed light on the

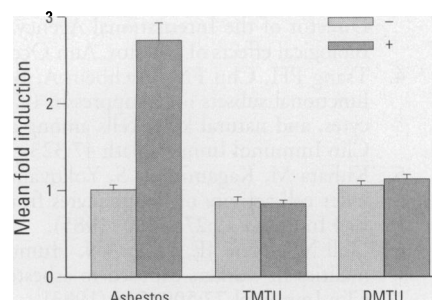


Figure 5. Inhibition of asbestos-induced IL-8 promoter-driven transcription by antioxidants. A549 cells were transfected with 1.5 μ g IL-8 Chloramphenicol acetyltransferase (CAT) reporter plasmid and 0.5 mg of β -actin luciferase control plasmid. The transfected cells were cultured with medium alone; asbestos (12.5 μ g/ml); asbestos in the presence of 10 mM TMTU or DMTU, or TMTU or DMTU alone. The activation of IL-8 promoter was determined by assaying the CAT concentration of cellular extracts prepared 26 to 28 hr after transfection. Transfection efficiencies were normalized by analyzing luciferase activity. The results are presented relative to CAT levels in unstimulated cells. Experiments were repeated a total of three times with representative data shown. Data adapted from Simeonova and Luster (125).

cellular and subcellular events responsible for asbestos-related immune dysfunction, these same studies have also shown the remarkable complexity in fiber-cell and cell-cell interactions involved in asbestos-related disease processes. However, key questions remain to be fully understood, including an understanding of the genetic predisposition of individuals with a tendency for immunologic hyperactivity to asbestos, the interactions between systemic and local immune changes, and the influence of complex mixtures of cytokines/growth factors on the pathogenesis of asbestos-induced lung disease. Nevertheless, the impressive data generated to date on asbestos-immune system interactions will likely aid in identifying the toxic and neoplastic properties of additional natural and synthetic fibers.

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